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DATE: Thursday, July 27, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	(groel or gro-el) same (groes or gro-es)	364
☐	L2	L1 same complex$	114
☐	L3	L2.ti,ab,clm.	6
☐	L4	(hsp$ or cpn$ or (stress near (protein or peptide polypeptide or poly-peptide))).ti,ab,clm.	3034
☐	L5	L4 same (complex$ or join$ or attach$ or bound or binding or associat$).ti,ab,clm.	857
☐	L6	L5 same (peptide or polypeptide or protein or urease or ure-a or ureb or ure-b or poly-peptide).ti,ab,clm.	626
☐	L7	L5 same (peptide or polypeptide or protein or urease or ure-a or ureb or ure-b or poly-peptide).ti,ab,clm.	626
☐	L8	L7 same (composition or pharmaceutic$ or vaccine or formulate or formulation or depot or adjuvant or carrier).ti,ab,clm.	246
☐	L9	l8 and (anthony.in. or calaco.in.)	3
☐	L10	l8 not l9	243
☐	L11	L10	243

END OF SEARCH HISTORY

L14: Entry 4 of 92 File: USPT Mar 28, 2006

DOCUMENT-IDENTIFIER: US 7018791 B1
TITLE: Binding complexes

Brief Summary Text (7):
The generally accepted role for GroEL is that it binds to exposed hydrophobic regions of polypeptides that are normally buried within the cores of soluble proteins. By binding to the exposed hydrophobic regions the GroEL prevents aggregation between the unfolded protein monomers themselves or other intracellular molecules. Following substrate binding to GroEL, cycles of ATP hydrolysis drive the progression of the bound substrate towards a folded or near folded state which is then released from the folding complex. GroEL appears to be able to bind to many denatured proteins by means of interaction with hydrophobic pockets or clefts on the surface of the GroEL, indeed GroEL is able to bind to some 50% of denatured cytosolic proteins (Viitanen et al, Protein. Sci. 1, 363 369, 1992), which suggests a broad specificity for hydrophobic regions in substrate proteins. GroEL mediated folding and release of many substrates is facilitated by the ring co-chaperonin GroES which caps the active cis side of the folding complex (Weissman et al, Cell 84, 481 490, 1996).

Brief Summary Text (8):
By analysis the Type II Chaperonin from eukaryotes, CCT, appears to be an wholly different molecule to GroEL for a number of obvious structural and less obvious mechanistic reasons. CCT is a heteropolymeric complex comprised of eight different subunits in each of two rings which exist as a double toroid structure, the eight subunits being encoded by eight different genes. CCT also appears to bind a far more restricted spectrum of partially folded substrates than GroEL. CCT appears to primarily interact with proteins of the cytoskeleton, namely actin and tubulin, and indeed there are some denatured soluble proteins which CCT will simply not bind (Melki and Cowan, Mol. Cell Biol. 14, 2895 2904, 1994). CCT, like GroEL, possesses ATPase activity and the ATPase domain on each CCT subunit is the region showing highest homology with GroEL. There is no GroES like co-chaperonin known for any of the type II chaperonins.

DOCUMENT-IDENTIFIER: US 20030099665 A1
TITLE: Chaperone and adhesin proteins; vaccines, diagnostics and method for treating infections

Summary of Invention Paragraph:
[0013] In one aspect the present invention relates to a vaccine for treating or preventing bacterial infections which utilizes as an immunogen a complex of a bacterial periplasmic chaperone protein with a bacterial adhesin protein. Preferably, the adhesin protein is a pilus adhesin protein. In one embodiment, the periplasmic chaperone protein and pilus adhesin protein are from E. coli; for example, a member selected from the complexes PapD/PapG and FimC/FimH.

Summary of Invention Paragraph:
[0014] In a particular aspect, the invention relates to vaccines formulated from type 1 pilus-associated adhesins (or from mannose-binding fragments thereof) or from complexes of chaperone proteins (including PapD-like chaperones) and pilus-associated adhesins for the treatment and/or prophylaxis of diseases caused by pathogenic species of gram-negative bacteria, such as Escherichia coli (E. coli). For example, it relates to treatment and/or prophylaxis of urinary tract infections caused by E. coli with vaccines formulated from at least one of (1) a fragment of the pilus-associated adhesin FimH that retains mannose binding capability (alone or complexed with its chaperone FimC), (2) the pilus-associated adhesin PapG protein complexed with its periplasmic chaperone protein PapD or (3) the full-length pilus-associated adhesin FimH (alone or in a complex with its chaperone protein FimC). This invention also relates generally to the use of heteropolymeric protein complexes to raise antibodies in non-human mammalian species useful, for example, as diagnostic reagents and vaccines.

Summary of Invention Paragraph:
[0019] In a further aspect, the present invention relates to the production of a periplasmic chaperone protein in a complex with an essentially full-length bacterial adhesin protein or appropriate fragment thereof in a recombinant host (in E. coli, another bacterial species, a bacterial species with one or more disabled proteases, or a non-bacterial production vector or host cell) or by synthesis or by recovering from a natural source. Even more preferable is the production of the periplasmic chaperone protein FimC complexed with essentially full-length FimH or a mannose binding analog or variant for use as a vaccine.

Brief Description of Drawings Paragraph:
[0022] FIGS. 1A and 1B show data results of immunoglobulin G (IgG) titer to FimHt adhesin and whole type 1 pili, respectively, up to 78 weeks post immunization with purified adhesin, adhesin-chaperone complex or whole type 1 pili, further described in Example 1.

Detail Description Paragraph:
[0025] It is an object of the present invention to utilize as immunogenic composition for a vaccine (or to produce antibodies for use as a diagnostic or as a passive vaccine) comprising a bacterial adhesin protein or a complex of a bacterial periplasmic chaperone protein and such a bacterial adhesin protein. In one embodiment, proteins (naturally or recombinantly produced, as well as functional analogs) from bacteria that produce type 1 pili are contemplated. Even more particularly, E coli proteins are contemplated.

Detail Description Paragraph:
[0026] A particularly preferred embodiment of such an immunogenic composition is for use as a vaccine (or as an immunogen for producing antibodies useful for diagnostics or vaccines) wherein the active component of the immunogenic composition is a member selected from mannose-binding fragments of FimH adhesin protein (alone or complexed with a periplasmic chaperone protein), the PapG adhesin protein complexed with its chaperone protein PapD or the full-length FimH adhesin protein (alone or

complexed with FimC). Of course, any adhesin assembled by the chaperone/usher pathway could be prepared and utilized as a vaccine according to the present invention.

Detail Description Paragraph:
[0030] In one aspect, the present invention is directed to an immunogenic composition comprising a purified complex of a periplasmic chaperone protein and a chaperone-binding protein. The chaperone-binding protein is maintained in the complex in an immunogenic form capable of inducing an immune response when appropriately introduced into a human or other mammalian species. Adhesins are suitable chaperone-binding proteins for use in these immunogenic compositions.

Detail Description Paragraph:
[0040] In addition to use as vaccines, such pili adhesin proteins, mannose-binding adhesin protein fragments, and complexes of periplasmic chaperone proteins and such pili adhesin proteins can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

Detail Description Paragraph:
[0045] The present invention provides for a recombinant production or synthesis of adhesin proteins of pilus-bearing bacteria adhesin proteins in the presence or absence of its chaperone protein for use as a vaccine (or as an immunogen to produce antibodies for diagnostic or therapeutic purposes). In particular an adhesin protein may be individually expressed or co-expressed with its corresponding periplasmic chaperone protein to make a complex of the co-expressed proteins. Preferably, the adhesin protein is a substantially full-length FimH protein, or an analog or derivative thereof which maintains mannose binding capability. In this regard cDNA, RNA and genomic sequences for such chaperone and adhesin proteins are known. See Tables 1 and 2, below.

Detail Description Paragraph:
[0076] Procedures for the isolation of a periplasmic chaperone protein complexed with an adhesin protein are known in the art, as an example see Jones et al., Proc. Natl. Acad. Sci. (USA) 90:8397-8401 (1993). Further, the individually expressed adhesin proteins may be isolated by recombinant expression/isolation methods that are well-known in the art. Typical examples for such isolation may utilize an antibody to the protein or to a His tag or cleavable leader or tail that is expressing as part of the protein structure.

Detail Description Paragraph:
[0091] The immunogenicity of purified adhesin, adhesin-chaperone complex or whole type 1 pili proteins were assessed by measuring immunoglobulin G (IgG) titer to FimHt adhesin (a naturally occurring FimH truncate corresponding to the NH.sub.2-terminal two-thirds of the FimH protein which was purified away from complexes of FimC and FimH (FimC-H)) and whole type 1 pili, respectively, up to 78 weeks post immunization.

Detail Description Paragraph:
[0092] C3H/HeJ mice, five mice per group were immunized on day 0 (primary immunization) [in Freund's adjuvant (CFA)] and booster immunization (week 4) [in incomplete Freund's adjuvant (IFA)] with one of the three antigens: purified adhesin (FimHt), adhesin-chaperone complex (FimC-H) or whole type 1 pili. Samples from individual mice treated identically were pooled for serological analysis and diluted 1:100 before serial dilution. Antibody responses were assessed by an ELISA with purified FimHt or whole pili as the capture antigens. Titers reflect the highest dilution of serum reacting twice as strongly as a comparable dilution of preimmune sera obtained from the same mice. The purity of the protein preparations of the capture antigens was 95% pure for whole type 1 pili and FimHt to 98 to 99% purity for FimC-H. In all cases the protein preparations were free of any lipopolysaccharide

contaminants. Data for immune responses of such mice to FimHt adhesin (FIG. 1A) and whole type 1 pili (FIG. 1B) of such mice are reported in FIGS. 1A and 1B as FimHt (squares), FimC-H (circles) or whole type 1 pili (triangles).

CLAIMS:

1. A vaccine against bacterial infections comprising a complex of a bacterial chaperone protein with either an adhesin protein or an immunogenic fragment of said adhesin protein.

17. A method for preventing and or treating UTIs in a host comprising immunizing said host with a member selected from the group consisting of: (a) a vaccine according to claim 1, and (b) at least one antibody raised against a complex of a bacterial chaperone protein with either an adhesin protein or an immunogenic mannose-binding fragment of said adhesin protein.

DOCUMENT-IDENTIFIER: US 20030054010 A1
TITLE: Molecular antigen array

Detail Description Paragraph:
[0428] Gene deletion studies proved that removal of the pilus chaperones leads to a total loss of piliation in P-pili and Type-1 pili (Lindberg, F, et al., J. Bacteriol. 171:6052-6058 (1989); Klemm, P., Res. Microbiol. 143:831-838 (1992); Jones, C. H., et al., Proc. Nat. Acad Sci. USA 90:8397-8401 (1993)). Periplasmic extracts of a .DELTA. fimC strain showed the accumulation of the main subunit FimA, but no pili could be detected (Klemm, P., Res. Microbiol. 143:831-838 (1992)). Attempts to over-express individual P-pilus subunits failed and only proteolytically degraded forms could be detected in the absence of PapD; in addition, the P-pilus adhesin was purified with the inner membrane fraction in the absence of the chaperone (Lindberg, F., et al., J. Bacteriol. 171:6052-6058 (1989)) However, co-expression of the structural pilus proteins and their chaperone allowed the detection of chaperone/subunit complexes from the periplasm in the case of the FimC/FimH complex as well as in the case of different Pap-proteins including the adhesin PapG and the main subunit PapA (Tewari, R., et al., J. Biol. Chem. 268:3009-3015 (1993); Lindberg, F., et al., J. Bacteriol. 171:6052-6058 (1989)). The affinity of chaperone/subunit complexes towards their assembly platform has also been investigated in vitro and was found to differ strongly (Dodsonet et al., Proc. Natl. Acad. Sci. USA 90:3670-3674 (1993)). From these results the following functions were suggested for the pilus chaperones:

L5: Entry 110 of 163 File: USPT Nov 15, 2005

DOCUMENT-IDENTIFIER: US 6964769 B2
TITLE: Molecular antigen array

Detailed Description Text (425):
Gene deletion studies proved that removal of the pilus chaperones leads to a total loss of piliation in P-pili and Type-1 pili (Lindberg, F., et al., J. Bacteriol. 171:6052-6058 (1989); Klemm, P., Res. Microbiol. 143:831-838 (1992); Jones, C. H., et al., Proc. Nat. Acad Sci. USA 90:8397-8401 (1993)). Periplasmic extracts of a .DELTA.fimC strain showed the accumulation of the main subunit FimA, but no pili could be detected (Klemm, P., Res. Microbiol. 143:831-838 (1992)). Attempts to over-express individual P-pilus subunits failed and only proteolytically degraded forms could be detected in the absence of PapD; in addition, the P-pilus adhesin was purified with the inner membrane fraction in the absence of the chaperone (Lindberg, F., et al., J. Bacteriol. 171:6052-6058 (1989)). However, co-expression of the structural pilus proteins and their chaperone allowed the detection of chaperone/subunit complexes from the periplasm in the case of the FimC/FimH complex as well as in the case of different Pap-proteins including the adhesin PapG and the main subunit PapA (Tewari, R., et al., J. Biol. Chem. 268:3009-3015 (1993); Lindberg, F., et al., J. Bacteriol. 171:6052-6058 (1989)). The affinity of chaperone/subunit complexes towards their assembly platform has also been investigated in vitro and was found to differ strongly (Dodson et al., Proc. Natl. Acad. Sci. USA 90:3670-3674(1993)). From these results the following functions were suggested for the pilus chaperones:

L5: Entry 102 of 163 File: USPT May 23, 2006

DOCUMENT-IDENTIFIER: US 7048756 B2
TITLE: System, method and apparatus for evaluating tissue temperature

PRIOR-PUBLICATION:

DOC-ID	DATE
US 20040122494 A1	June 24, 2004

Brief Summary Text (31):
The host immune system can be activated against infectious disease by heat shock protein chaperoned peptides in a manner similar to the effect seen against metastatic tumors. Heat shock proteins chaperoning peptides derived from both viral and bacterial pathogens have been shown to be effective at creating immunity against the infectious agent. For infectious agents for which efficacious vaccines are not currently available (especially for intracellular pathogens e.g. viruses, Mycobacterium tuberculosis or Plasmodium) HSP chaperoned peptides may be useful for the development of novel vaccines. It is expected that purified HSP chaperoned peptides (e.g. gp96 complexes) used as vaccines for diseases caused by highly polymorphic infectious agents would be less effective against genetically distinct pathogen populations. For a summary of past work on HSP vaccines against infectious agents, see generally: (26) Neiland, Thomas J. F., M. C. Agnes A. Tan, Monique Monnee-van Muijen, Frits Koning, Ada M. Kruisbeek, and Grada M. van Bleek, "Isolation of an immunodonminant viral peptide that is endogenously bound to stress protein gp96/GRP94." Proc. Nat'l Acad. Sci. USA, 93: 6135 6139 (1996). (27) Heikema, A., Agsteribbe, E., Wilschut, J., Huckriede, A., "Generation of heat shock protein-based vaccines by intracellular loading of gp96 with antigenic peptides." Immunology Letters, 57: 69 74 (1997). (28) Zugel, U., Sponaas, A. M., Neckermann, J., Schoel, B., and Kaufmann, S. H. E., "gp96-Peptide Vaccination of Mice Against Intracellular Bacteria." Infection and Immunity, 69: 4164 4167 (2001). (29) Zugel, U., and Kaufmann, S. H. E., "Role of Heat Shock Proteins in Protection from and Pathogenesis of Infectious Diseases." Clinical Microbiology Reviews, 12: 19 39 (1999).

☐ 1. 20040029806. 06 Aug 03. 12 Feb 04. Medicament. Burnie, James P., et al. 514/12; 530/350 530/388.1 A61K038/17 C07K014/47 C07K016/18.

☐ 2. 20030180285. 07 Oct 02. 25 Sep 03. Treatment of fungal infections with polyene or beta glucan synthase inhibitor anti-fungals combined with anti hsp90 antibodies. Burnie, James P. 424/130.1; 514/27 A61K039/395 A61K031/7048.

☐ 3. 6040148. 22 Mar 95; 21 Mar 00. Corynebacterial stress proteins. Burnie; Peter James. 435/7.32; 424/166.1 424/245.1 530/325 530/326 530/327 530/350 530/387.1 530/387.9 530/388.2 530/388.4 530/402 530/825. G01N033/569 C07K014/34 C07K007/00 A61K039/05 .

☐ 4. 5985277. 19 Jun 97; 16 Nov 99. Antibodies to bacterial stress proteins. Burnie; Peter James. 424/139.1; 424/141.1 424/151.1 424/166.1 530/387.9 530/388.4 530/389.5 536/23.7. A61K039/395 A61K039/40 C07K016/00 C07H021/04 .

☐ 5. 5777083. 10 Apr 95; 07 Jul 98. Stress protein epitopes. Burnie; James Peter, et al. 530/387.3; 435/320.1 435/7.31. C12P021/08 .

☐ 6. 5686248. 28 Jun 96; 11 Nov 97. Fungal stress proteins. Burnie; James Peter, et al. 435/6; 435/91.1 435/91.2 435/921 435/922 536/23.1 536/23.7 536/23.74 536/24.32 536/24.33. C12Q001/68 C12P019/34 C12N001/00 C07H021/04 .

☐ 7. 5541077. 13 Dec 94; 30 Jul 96. Fungal stress proteins. Burnie; James P., et al. 435/7.31; 435/7.92 435/7.95 436/530 436/534 436/815 530/387.9 530/388.5 530/389.1. G01N033/569 C07K016/14 .

☐ 8. 5288639. 14 Mar 91; 22 Feb 94. Fungal stress proteins. Burnie; James P., et al. 435/320.1; 435/921 435/922 435/924 530/300 530/327 530/328 530/329 530/330 530/350 530/371 530/806 530/823 536/23.74. C12N015/31 C12N015/63 C07K005/00 C07K007/00 C07K013/00 .

The Structure of the GroEL/GroES/ADP Complex

Z. Xu, A. Horwich and P. Sigler (Yale University)

The structure of the molecular chaperone GroEL in complex with a partner assembly GroES in the presence of bound ADP, was determined to a resolution of 3.0 Å using x-ray diffraction data collected at beamline X25 by Z. Xu, A. Horwich, and P. Sigler of Yale U. and the Howard Hughes Medical Institute (HHMI)[1]. The GroEL/GroES complex facilitates the folding of other proteins, and how this is accomplished is an important problem in modern biology. GroEL consists of two back-to-back 7-fold rotationally symmetric rings, that enclose two large, non-contiguous central cavities in which an unfolded protein can be bound. GroES consists of a single 7-fold rotationally symmetric ring, with mobile loops extending from its rim. In the presence of the nucleotide ATP, GroEL and GroES interact via these loops to form an asymmetrical GroEL/GroES complex. The x-ray diffraction data show that the overall structure of this complex undergoes a substantial change upon the binding of the nucleotide, arising from movements of the domain components of one of the GroEL rings (**Figure B-1**). This creates an enlarged cavity from which the bound protein can be released, upon dissociation of the GroES ring from the GroEL. The diffraction data also provide insight to the binding of the nucleotide to the complex. The crystals of this complex have a very large unit cell and diffract x-rays very weakly and anisotropically, and moreover are relatively small. Access to the high beam intensity of X25 was mandatory in order to determine its structure to such high resolution. ■

[1] Z. Xu, A. Horwich, and P. Sigler, *Nature* 388, 741-750, (1997).

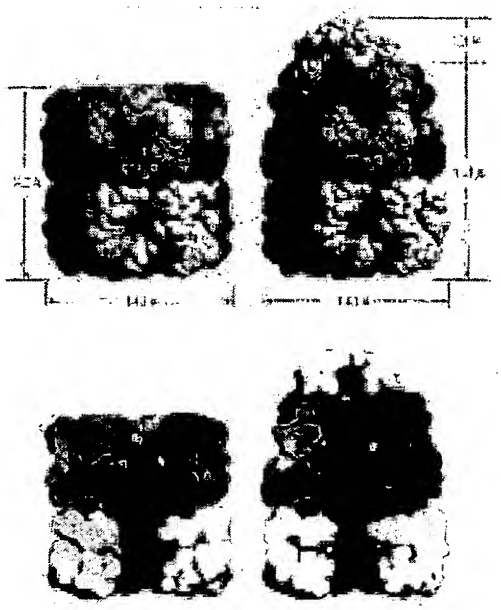

Figure B-1: Overall architecture and dimensions of GroEL and GroEL-GroES-$(ADP)_7$. Van der Waals space-filling models (6 Å spheres around Cα) of GroEL (**left**) and GroEL-GroES-$(ADP)_7$ (**right**). Upper panels are outside views, showing outer dimensions; lower panels show the insides of the assemblies and were generated by slicing off the front half with a vertical plane that contains the cylindrical axis. Various colors are used to distinguish the subunits of GroEL in the upper ring, with shading indicating domains; dark hue, equatorial domain; medium hue, apical domain; lightest hue, intermediate domain. The lower GroEL ring is uniformly yellow. GroES is uniformly gray.

GroEL-GroES Complex from E. coli

This huge complex (21 chains, 58800 heavy atoms) acts as a "foldase", that is, an enzyme that catalyzes refolding of proteins that have been denatured by say heat. The process is ATP dependent. This form of the complex has 7 ATPs bound - see a red or orange group in the middle of teach of proteins in the central protein ring.

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☐	L1	burnie.in. and (cpn or hsp or stress or (heat near shock))	19
☐	L2	l1 and complex$	8
☐	L3	chaperon$ near5 complex$	527
☐	L4	L3 and (bacter$ or prokaryot$ or procaryot$ or coli or salmonella or pathogen$)	458
☐	L5	L3 same (purif$ or isolat$ or chromatog$ or vaccin$ or composition or pharmaceutic$)	163

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 20020034515 A1
TITLE: Proteus mirabilis-based vaccine

Detail Description Paragraph:
[0136] Studies by Langermann et. al. ((1997) Science 276:607-611, incorporated herein by reference) showed that FimH-adhesin-based systemic vaccination prevented type-l-piliated E. coli colonization in mice. MrpH-MrpD (adhesin-chaperone) complexes were prepared in order to express the MrpH adhesin in its native conformation as was done for FimC-FimH conjugates for an E. coli type 1 fimbrial vaccine Langermann et. al. (1997) supra. To accomplish this, MrpH was copurified with its chaperone protein MrpD which, by analogy to homologs in the E. coli P fimbriae system, capped the protein in the periplasmic space and prevented polymerization until the adhesin protein was translocated across the outer 1 membrane.

DOCUMENT-IDENTIFIER: US 20020146428 A1
TITLE: Treatment or prophylaxis of diseases caused by pilus-forming bacteria

Summary of Invention Paragraph:
[0066] In step 1c) the periplasmic molecular chaperone or the analogue thereof being bound to the pilus subunit or the equivalent thereof may be detected by separation of pilus subunit/chaperon complexes (e.g. by ultracentrifugation, ultrafiltration, liquid chromatography, such as size exclusion chromatography, or electrophoresis). Described below is a method relying on the changes in fluorescence of a short PapG fragment when this fragment is bound to PapD. This method is a preferred assay in the method of the invention.

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DATE: Thursday, July 27, 2006

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☐	L1	(groel or gro-el) same (groes or gro-es)	364
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☐	L4	(hsp$ or cpn$ or (stress near (protein or peptide polypeptide or poly-peptide))).ti,ab,clm.	3034
☐	L5	L4 same (complex$ or join$ or attach$ or bound or binding or associat$).ti,ab,clm.	857
☐	L6	L5 same (peptide or polypeptide or protein or urease or ure-a or ureb or ure-b or poly-peptide).ti,ab,clm.	626
☐	L7	L5 same (peptide or polypeptide or protein or urease or ure-a or ureb or ure-b or poly-peptide).ti,ab,clm.	626
☐	L8	L7 same (composition or pharmaceutic$ or vaccine or formulate or formulation or depot or adjuvant or carrier).ti,ab,clm.	246
☐	L9	l8 and (anthony.in. or calaco.in.)	3
☐	L10	l8 not l9	243
☐	L11	L10	243
	DB=USPT; PLUR=YES; OP=OR		
☐	L12	(groel or gro-el) same (groes or gro-es)	157
☐	L13	L12 same (complex$ or join$ or attach$ or bound or binding or associat$)	99
☐	L14	L13 same (protein or peptide polypeptide or poly-peptide)	92

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L14: Entry 73 of 92 File: USPT Jun 19, 2001

DOCUMENT-IDENTIFIER: US 6248330 B1
** See image for Certificate of Correction **
TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Detailed Description Text (39):
The invention also concerns monoclonal or polyclonal antibodies to the Hsps or fragments thereof, particularly to the HspA (SEQ ID NO:29) and/or HspB (SEQ ID NO:30) protein illustrated in FIG. 6. Polypeptides having at least 75%, and preferably at least 80%, or 90%, homology with the Hsps may also be used to induce antibody formation. These antibodies may be specific for the Helicobacter pylori chaperonins or, alternatively, they may cross-react with GroEL-like proteins or GroES-like proteins from bacteria other than Helicobacter, depending upon the epitopes recognized. FIG. 7 shows the homologous regions of HspA (SEQ ID NO:29) and HspB (SEQ ID NO:30) with GroES-like proteins (SEQ ID NOS:31-35) and GroEL-like proteins (SEQ ID NOS:31-35), respectively, from various bacteria. Particularly preferred antibodies are those specific for either the HspA or HspB chaperonins or those specifically recognizing the HspA C-terminal sequence having the metal binding function. Again, use of specific fragments for the induction of the antibodies ensures production of Helicobacter-specific antibodies.

Detailed Description Text (170):
A homolog of the heat shock proteins (Hsps) of the GroEL class, reported to be closely associated with the urease of Helicobacter pylori (a nickel metalloenzyme), has recently been purified from H. pylori cells by Dunn et al., and Evans et al. (Infect. Immun. 60:1946, 1992, 1946 and 2125, respectively). Based on the reported N-terminal amino acid sequence of this immunodominant protein, degenerate oligonucleotides were synthesized in order to target the gene (HspB (SEQ ID NO:28)) encoding the GroEL-like protein in the chromosome of H. pylori strain 85P. Following gene amplification, a 108-base pair (bp)-fragment encoding the 36 first amino acids of the HspB protein (SEQ ID NO:30) was purified, and used a probe to identify in the H. pylori genomic bank a recombinant cosmid harboring the entire HspB encoding gene (SEQ ID NO:28). The HspB gene (SEQ ID NO:28) was mapped to a 3.15 kilobases (kb) BglII restriction fragment of the pILL684 cosmid. The nucleotide sequence of that fragment subcloned into the pILL570 plasmid vector (pILL689) revealed the presence of two open reading frames (OFRS) designated HspA and HspB, the organization of which was very similar to be groESL bicistronic operons of other bacterial species. HspA (SEQ ID NO:30) and HspB (SEQ ID NO:28) encode polypeptides of 118 and 545 amino acids, respectively, corresponding to calculated molecular masses of 13.0 and 58.2 kilodaltons (kDa), respectively. Amino acid sequence comparison studies revealed i) that the H. pylori HspA (SEQ ID NO:29) and HspB (SEQ ID NO:30) protein were highly similar to their bacterial homologs; ii) that the HspA (SEQ ID NO:29) H. pylori protein features a striking motif at the carboxyl terminus that other bacterial GroEs-homologs lack; this unique motif consists of a series of eight histidine residues resembling metal binding domain, such a nickel binding. Surprisingly, immediately upstream of the gene cluster an IS5 insertion element was found that was absent in the H. pylori genome, and was positively selected during the cosmid cloning process. The IS5 was found to be involved in the expression of the HspA (SEQ ID NO:29)and HspB genes in pILL689. The

expression of the HspA and HspB (SEQ ID NO:30) proteins from the pILL689 plasmid was analyzed in minicell-producing strain. Both polypeptides were shown to be constitutively expressed in the E. coli cells. When the pILL689 recombinant plasmid was introduced together with the H. pylori urease gene cluster into an E. coli host strain, an increase of urease activity was observed suggesting a close interaction between the heat shock proteins and the urease enzyme. Supporting the concept of a specific function for the HspA chaperone, was the fact that whereas a single HspB copy was found in the H. pylori genome, two copies of the HspA were found in the genome, one linked to the HspB gene and one unlinked to the HspB gene. Attempts to construct isogenic mutants of H. pylori in the HspA and the HspB gene were unsuccessful suggesting that these genes are essential for the survival of the bacteria.

Detailed Description Text (198):
The deduced amino acid sequences of H. pylori HspA (SEQ ID NO:29) and HspB (SEQ ID NO:30) were compared to several amino acid sequences of Hsps of the GroES and GroEL class (FIG. 7). HspB exhibited high homology at the amino acid level with the Legionella pneumophila HtpB protein (82.9% of similarities), with the Escherichia coli GroEL protein (81.0% of similarities), with the Chlamydia psittaci or C. trachomatis HypB protein (79.4% of similarities), with Clostridium perfringens Hsp60 protein (80.7% of similarities), and to a lesser extent to the GroEL-like proteins of Mycobacterium. However, like almost all the GroEL homologs, H. pylori HspB (SEQ ID NO:30) demonstrated the conserved carboxyl-terminus glycine-methionine motif (MGGMGGMGGMGGMM (SEQ ID NO:12)), which was recently shown to be dispensable in the E. coli GroEL chaperonin. The degree of homology at the amino acid level between the H. pylori HspA (SEQ ID NO:29) protein and the other GroES-like proteins (SEQ ID NOS:36-40) is shown in FIG. 7. The alignment shown features a striking motif at the carboxyl terminus of the H. pylori HspA (SEQ ID NO:29) protein that other bacterial GroES-homologs lack. This unique highly charged motif consists of 27 additional amino acids capable of forming a loop between two double cysteine residues; of the 27 amino acids, 8 are histidine residues highly reminiscent of a metal binding domain.

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DOCUMENT-IDENTIFIER: US 6410262 B1
TITLE: Secretion factors for gram-positive microorganisms genes encoding them and methods of using it

Brief Summary Text (7):
For instance, SecB, GroEL/GroES and DnaK/DnaJ are the presently known chaperones in the export pathway of E. coli. For the productive binding of precursor proteins to translocation sites in the cytoplasmic membrane SecA is needed. SecA, a protein of which cytoplasmic, peripheral as well as integral membrane forms have been detected, has an ATPase activity which mediates the initial channelling of precursor proteins into the export pathway.

Detailed Description Text (3):
Because homologues of SecA (Sadaie et al. 1991), SecE (Jeong et al. 1993), SecY (Su et al. 1990), and Lep (Van Dijl et al. 1992) have been identified in B. subtilis, it is suggested that signal peptide-dependent protein secretion in B. subtilis utilizes a Sec-pathway that is similar to that of E. coli. So far SecB, which is considered to be the major chaperone in E. coli, seems to be the only chaperone which has a direct binding affinity for SecA and so contributes to the accurate targeting of the preprotein-SecB complex to the membrane bound translocase. The SecB protein is needed for only a subset of the envelope proteins so SecB independent proteins will enter the Sec-pathway with the aid of helper proteins like GroEL/GroES, DnaK/DnaJ or other proteins like SRP. In eukaryotic organism SRP mainly is responsible for the translocation across the ER membrane. Recently more evidence has become available of the existence of an SRP mediated secretion route in bacteria. Because the eukaryotic pathway has probably evolved from the bacteria it is thinkable that said proteins are also dependent on this pathway when said proteins are expressed in bacteria like Bacillus. Thus optimisation of this particularly pathway in Bacillus will be more profitable for heterologous (eukaryotic) proteins secretion than the optimisation of the well known sec-pathway. This invention relates to the cloning of the Bacillus ftsY gene and its effect after (over)-expression, alone or in combination with other members of the bacterial SRP, upon heterologous proteins.

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L14: Entry 71 of 92 File: USPT Jul 10, 2001

US-PAT-NO: 6258359
DOCUMENT-IDENTIFIER: US 6258359 B1
**** See image for Certificate of Correction ****

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 424/141.1; 424/150.1, 424/163.1, 424/164.1, 530/350, 530/388.1, 530/388.2, 530/388.4

CLAIMS:

What is claimed is:

1. A monoclonal antibody directed against a polypeptide selected from the group consisting of Helicobacter HspA consisting of SEQ ID NO: 29, HspB consisting of SEQ ID NO: 30, and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment.

2. A monoclonal antibody directed against a fragment of HspA of Helicobacter pylori consisting of the following sequence: GSCCHTGNHDHKAKEHEACCHDHKKH (SEQ ID NO: 1).

3. A monoclonal antibody directed against a fragment of HspA of Helicobacter pylori, wherein said HspA of Helicobacter pylori consists of SEQ ID NO: 29, and wherein said fragment has at least 6 consecutive amino acids from the following amino acid sequence:

GSCCHTGNHDHKAKEHEACCHDHKKH (SEQ ID NO: 1) and is recognized by an antibody directed against the full length HspA polypeptide.

4. A composition comprising purified monoclonal antibodies directed against the following peptides:

a) at least one urease polypeptide of Helicobacter felis or Helicobacter pylori selected from the group consisting of UreA, UreB, UreE, UreF, UreG, UreH, UreI, and fragments thereof, wherein said fragment is also recognized by

an antibody directed against the full length polypeptide corresponding to that fragment; and

b) at least one polypeptide of Helicobacter felis or Helicobacter pylori selected from the group consisting of HspA (SEQ ID NO: 29), HspB (SEQ ID NO: 30), and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment.

5. The composition of claim 4, wherein said urease polypeptide is UreA of Helicobacter pylori.

6. The composition of claim 4, wherein said urease polypeptide is UreB of Helicobacter pylori.

7. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of Helicobacter pylori.

8. The composition of claim 4, wherein said urease polypeptide is UreA of Helicobacter felis.

9. The composition of claim 4, wherein said urease polypeptide is UreB of Helicobacter felis.

10. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of Helicobacter felis.

11. A method of making an antibody comprising the steps of

providing at least one polypeptide of Helicobacter selected from the group consisting of

(a) Helicobacter heat shock polypeptide HspB (SEQ ID NO: 30),

(b) Helicobacter heat shock polypeptide HspA (SEQ ID NO: 29), and

(c) fragments thereof that are recognized by monoclonal antibodies directed against the full length polypeptide corresponding to that fragment;

immunizing a host animal with said polypeptide; and

purifying said antibody from serum of said host animal.

12. A method of making a monoclonal antibody comprising the steps of

immunizing a host animal with at least one polypeptide of Helicobacter selected from the group consisting of

(a) Helicobacter heat shock polypeptide HspB (SEQ ID NO: 30),

(b) Helicobacter heat shock polypeptide HspA (SEQ ID NO: 29), and

(c) fragments thereof that are recognized by monoclonal antibodies directed against the full length polypeptide corresponding to that fragment;

obtaining a splenocyte from the immunized host animal;

fusing said splenocyte with a myeloma cell;

screening for a hybridoma producing the monoclonal antibody; and

purifying said monoclonal antibody.

13. A method of using an antibody of any one of claims 1, 2, or 3 to induce an immunogenic response in a host animal comprising injecting said antibody into said host animal.

14. A monoclonal antibody directed against a polypeptide encoded by SEQ ID NO: 19.

15. The antibody of claim 14, wherein the polypeptide comprises SEQ ID NO: 20.

16. The antibody of claim 14, wherein the polypeptide comprises SEQ ID NO: 21.

17. A monoclonal antibody directed against an HspA polypeptide encoded by SEQ ID NO: 27.

18. The antibody of claim 17, wherein the polypcptide comprises SEQ ID NO: 29.

19. A purified antibody directed against a UreI polypeptide encoded by SEQ ID NO: 41.

20. The antibody of claim 19, wherein the polypeptide is SEQ ID NO: 42.

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DOCUMENT-IDENTIFIER: US 6433141 B1
TITLE: Purified heat shock protein complexes

Detailed Description Text (13):
Members of the hsp60 family include hsp60, hsp65, rubisco binding protein, and TCP-1 in eukaryotes; and GroEl/GroES in prokaryotes; Mif4, and TCPlalpha and beta in yeast.

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L14: Entry 30 of 92 File: USPT Feb 17, 2004

DOCUMENT-IDENTIFIER: US 6692951 B2
TITLE: Secretion factors for gram-positive micoorganisms, genes encoding them and methods of using it

Brief Summary Text (7):
For instance, SecB, GroEL/GroES and DnaK/DnaJ are the presently known chaperones in the export pathway of E. coli. For the productive binding of precursor proteins to translocation sites in the cytoplasmic membrane SecA is needed. SecA, a protein of which cytoplasmic, peripheral as well as integral membrane forms have been detected, has an ATPase activity which mediates the initial channelling of precursor proteins into the export pathway.

Detailed Description Text (3):
Because homologues of SecA (Sadaie et al. 1991), SecE (Jeong et al. 1993), SecY (Su et al. 1990), and Lep (Van Dijl et al. 1992) have been identified in B. subtilis, it is suggested that signal peptide-dependent protein secretion in B. subtilis utilizes a Sec-pathway that is similar to that of E. coli. So far SecB, which is considered to be the major chaperone in E. coli, seems to be the only chaperone which has a direct binding affinity for SecA and so contributes to the accurate targeting of the preprotein-SecB complex to the membrane bound translocase. The SecB protein is needed for only a subset of the envelope proteins so SecB independent proteins will enter the Sec-pathway with the aid of helper proteins like GroEL/GroES, DnaK/DnaJ or other proteins like SRP. In eukaryotic organism SRP mainly is responsible for the translocation across the ER membrane. Recently more evidence has become available of the existence of an SRP mediated secretion route in bacteria. Because the eukaryotic pathway has probably evolved from the bacteria it is thinkable that said proteins are also dependent on this pathway when said proteins are expressed in bacteria like Bacillus. Thus optimisation of this particularly pathway in Bacillus will be more profitable for heterologous (eukaryotic) proteins secretion than the optimisation of the well known sec-pathway. This invention relates to the cloning of the Bacillus ftsY gene and its effect after (over)-expression, alone or in combination with other members of the bacterial SRP, upon heterologous proteins.

DOCUMENT-IDENTIFIER: US 6677139 B1
TITLE: Methods for production of proteins in host cells

Brief Summary Text (6):
Several E.coli proteins have been shown to exhibit chaperone activity: the 60 kDa heat shock protein (Hsp60) GroEL, a chaperonin and the smaller accessory protein GroES (10 kDa); the DnaK (Hsp70), DnaJ and GrpE complex; and the Clp system. Georgiou et al. supra. GroEL consists of 14 subunits which are arranged in two heptameric rings stacked back to back. The central cavity of the cylinder accepts unfolded substrate polypeptides in the conformation of a collapsed intermediate. GroEL interacts with GroES, a single heptameric ring that binds asymetrically to GroEL, capping one opening of the cylinder. GroES coordinates the ATP hydrolysis by GroEL with productive folding (Mayhew, M et al., Nature vol. 379:420-426.)

Brief Summary Text (8):
Landry, S. et al. (1993, Nature 364:255-258) disclose a polypeptide loop of the GroES/GroEL complex and Altamirano et al. (1997, Proc. Natl. Acad. Sci. USA, 94:3576-3578) disclose the use of immobilized fragments of the GroEL chaperonin in chromatography.

Brief Summary Text (15):
There are several well characterized chaperonin systems known in the art having two or more interacting partners, for example, Hsp60 and Hsp10 (GroEL/GroES); Hsp70 and Hsp40 and GrpE (DnaK/DNAJ/GrpE); ClipA/X and ClipP; Hsp90 and Hsp70 and other factors; TriC and other factors. The present invention encompasses chaperonin binding domains obtainable from these systems as long as the chaperonin binding domain is capable of binding to a chaperonin with an affinity of between about 10.sup.-2 and 10.sup.-8 Kd. In one embodiment, the chaperonin binding domain has the sequence as shown in SEQ ID NO: 3 through SEQ ID NO: 40. In yet another embodiment, the chaperonin binding domain is obtainable from the GroES co-chaperonin and said chaperonin is the GroEL chaperonin. In another embodiment, the binding domain comprises the amino acid sequence EVETKSAGGIVLTGSAAA(SEQ ID NO:2), In a further embodiment, the binding domain comprises a variation of the sequence EVETKSAGGIVLTGSAAA(SEQ ID NO:2), said variant being capable of binding to GroEL chaperonin with an affinity of 10.sup.-2 to 10.sup.-8 Kd. The present invention also provides expression vectors and host cells comprising a chaperonin protein binding domain.

Drawing Description Text (6):
FIG. 5 shows the design of a linker for attaching the GroEL-binding loop of GroES to proteins. Oligonucleotides (SEQ ID NOS:44 and 42) matching the two sequences are shown above were synthesized chemically, annealed to generate the duplex DNA fragment, and cloned into appropriate vectors. Linkage to a gene via the EcoRI overhang generates a protein that is 20 (1905) Daltons longer. The amino acid sequence provided in this Figure corresponds to SEQ ID NO:43.

Detailed Description Text (3):
The in vivo cellular processes of protein folding and assembly are controlled by molecular mechanisms associated with molecular chaperones such as chaperonins. As used herein, the term "chaperonin" refers to those molecules including heat shock proteins Hsp60 and like proteins that are expressed in any organism which are associated with protein folding. The present invention encompasses any chaperonin from any microbial source, virus or bacteriophage including the chaperonin systems, Hsp60 and Hsp10 (GroEL/GroES); Hsp70 and Hsp40 and GrpE (DnaK/DNAJ/GrpE); ClipA/X and ClipP; Hsp90 and Hsp70; and TriC. In a preferred embodiment, the chaperonin binding domain and the chaperonin are obtainable from the heat shock protein 60 (HSP60) class of proteins. Other chaperonins include mammalian or yeast HSP68, HSP70, HSP72, HSP73, clathrin uncoating ATPase, IpG heavy chain

binding protein (BiP), glucose-regulated proteins 75, 78, and 80 (GRP75, GRP78, and GRP80), HSC70, and yeast KAR2, BiP, SSA1-4, SSB1, SSD1 and the like. Chaperone proteins which can increase protein secretion also include enzymes which catalyze covalent modification of proteins, such as mammalian or yeast protein disulfide isomerase (PDI), prolyl-4-hydroxylase B-subunit, ER p59, glycosylation site binding protein (GSBP) and thyroid hormone binding protein (T3BP).

DOCUMENT-IDENTIFIER: US 6967088 B1
TITLE: Soluble recombinant botulinum toxin proteins

Detailed Description Text (726):
Co-overexpression of the E. coli GroEL/GroES folding chaperones in a cell expressing a recombinant foreign protein has been reported to enhance the solubility of some foreign proteins that are otherwise insoluble when expressed in E. coli [Gragerouu et al. (1992) Proc. Natl. Acad. Sci. USA 89:10344]. The improvement in solubility is thought to be due to chaperone-mediated binding and unfolding of insoluble denatured proteins, thus allowing multiple attempts for productive refolding of recombinant proteins. By overexpressing the chaperones, the unfolding/refolding reaction is driven by excess chaperone, resulting, in some cases, in higher yields of soluble protein.

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